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Coronary Artery Disease

Interleukin-10 Serum Levels and Systemic Endothelial Vasoreactivity in Patients With Coronary Artery Disease

Stephan Fichtlscherer, MD, Susanne Breuer, MD, Christopher Heeschen, MD, Stefanie Dimmeler, PhD, Andreas M. Zeiher, MD

Frankfurt, Germany

OBJECTIVES Because the endothelium is a major target for inflammatory cytokines, we investigated whether elevated interleukin (IL)-10 serum levels are associated with improved endothelial vasoreactivity in patients with coronary artery disease (CAD).

BACKGROUND Chronic inflammation plays a pivotal role in the progression of atherosclerosis. Interleukin-10 is an anti-inflammatory cytokine that exerts important protective effects on atherosclerotic lesion development in experimental animals.

METHODS Vasoreactivity was assessed in 65 male patients with documented CAD by measuring endothelium-dependent (acetylcholine [ACh] 10 to 50 μg/min) and endothelium-independent (sodium nitroprusside [SNP] 2 to 8 μg/min) forearm blood flow (FBF) responses using venous occlusion plethysmography.

RESULTS Serum levels of IL-10 were significantly correlated with ACh-induced FBF responses ($r = 0.31$, $p < 0.02$), but not with SNP responses. Importantly, if IL-10 serum levels were increased in patients with elevated C-reactive protein (CRP) levels, no impairment of ACh-stimulated FBF response was observed. On multivariate analysis, including low-density lipoprotein cholesterol, smoking, hypertension, diabetes, clinical status of the patients, and statin and/or angiotensin-converting enzyme inhibitor treatment, only IL-10 ($p < 0.02$) and CRP serum levels ($p < 0.02$) were significant independent predictors of ACh-induced FBF responses.

CONCLUSIONS Thus, increased IL-10 serum levels are associated with improved systemic endothelial vasoreactivity in patients with elevated CRP serum levels, demonstrating that the balance between pro- and anti-inflammatory mediators is a major determinant of endothelial function in patients with CAD. (J Am Coll Cardiol 2004;44:44–9) © 2004 by the American College of Cardiology Foundation

Atherosclerosis is currently regarded as a chronic inflammatory disease of the vascular wall (1). Systemic markers of inflammation have been shown to be of significant prognostic relevance for assessing the risk of atherosclerotic disease progression (2). Specifically, serum levels of the acute-phase reactant C-reactive protein (CRP) provide important information for risk assessment in healthy volunteers, patients at risk, as well as patients with established coronary artery disease (CAD) (3,4). However, data are sparse with respect to the clinical relevance of anti-inflammatory mediators to modulate inflammatory processes of the vascular wall and disease progression. In a recent study, we could demonstrate that elevated serum levels of the anti-inflammatory cytokine interleukin (IL)-10 are not only predictive of a better clinical outcome after acute coronary syndromes (ACS), but also abrogate the increased risk associated with elevated CRP serum levels (5).

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The vascular endothelium is a major target for inflammatory mediators. Indeed, elevated CRP levels are associated with impaired endothelial vasodilator function of both the coronary and systemic circulation (6,7). Moreover, impaired endothelial function not only correlates with the presence of classic risk factors for CAD (8), but also is predictive of future cardiovascular events in patients at risk (9–14). Experimentally, IL-10 was shown to protect endothelial function after an acute inflammatory stimulus by limiting increases in superoxide generation within the vascular wall (15). Therefore, the present study was designed to test the hypothesis that elevated serum levels of the anti-inflammatory cytokine IL-10 are associated with enhanced endothelial vasodilator function and may counteract the impairment in endothelial function associated with elevated CRP serum levels in patients with established CAD.

METHODS

Patients. A total of 65 male patients were recruited from the patient population undergoing diagnostic coronary an-

From the Department of Internal Medicine IV, Division of Cardiology, Johann W. Goethe University, Frankfurt, Germany. This study was supported by the Deutsche Forschungsgemeinschaft SFB 553, Project C5.

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Abbreviations and Acronyms

ACE	=	angiotensin-converting enzyme
ACh	=	acetylcholine
ACS	=	acute coronary syndrome
CAD	=	coronary artery disease
CRP	=	C-reactive protein
FBF	=	forearm blood flow
HDL	=	high-density lipoprotein
IL	=	interleukin
LDL	=	low-density lipoprotein
NO(S)	=	nitric oxide (synthase)
SNP	=	sodium nitroprusside

giography or interventional procedures during ACS. Because vascular reactivity and inflammatory markers may vary with sex hormone fluctuations, female patients were excluded from the study. Forty-nine patients had stable CAD for at least three months before forearm blood flow (FBF) measurements. Sixteen patients were studied within five days (4.8 ± 1.9 days) of an episode of unstable angina (Braunwald class IIIb), defined as angina at rest with ST-segment alterations. The presence of CAD in patients with stable CAD, as well as identification of the culprit lesion in patients with unstable CAD, was documented by coronary angiography. Echocardiography was performed in all patients in order to exclude patients with an impaired left ventricular ejection fraction (<50%). Because myocardial necrosis may induce an increase in CRP serum levels unrelated to vascular inflammation, patients with troponin T levels >0.2 ng/ml were excluded. In addition, patients with any evidence of inflammatory or malignant diseases were excluded. Screening tests were performed by evaluation of the patients' history and white blood cell count, and, if required, a urinary test or chest X-ray was performed. All patients were taking long-term aspirin (100 mg/day) and beta-blocker therapy. For FBF measurements, vasoactive medications, such as calcium channel blockers, angiotensin-converting enzyme (ACE) inhibitors, and long-acting nitrates, were withheld for at least 24 h before the study, whereas aspirin, beta-blockers, and statins were not discontinued.

The clinical characteristics of the patient population are summarized in Table 1. Of the eight patients with diabetes, three were treated with insulin and five received oral antidiabetic agents. There were no significant differences in medical treatment between patients with stable and those with unstable CAD.

The study protocol had been approved by the ethics committee of the J. W. Goethe University. All patients gave written, informed consent.

Study protocol. The FBF measurements were performed as previously described (7). In brief, FBF measurements were performed in the morning in a quiet, temperature-controlled room at 22°C (72°F). Patients were asked to refrain from drinking alcohol or caffeine and from smoking for 12 h before the examination. Under local anesthesia

Table 1. Baseline Clinical Characteristics of the Study Population (n = 65)

Characteristics	
Age (yrs)	53.8 ± 4.8
Total serum cholesterol (mg/dl)	195.2 ± 44.1
LDL cholesterol (mg/dl)	119.5 ± 38.4
HDL cholesterol (mg/dl)	48.2 ± 14.5
Mean arterial BP (mm Hg)	93.6 ± 14.7
Diabetes mellitus	8 (12%)
Current smoking	17 (26%)
Hypertension	29 (44%)
Unstable angina	16 (25%)
IL-10 serum levels (pg/ml)	2.4 ± 2.1
CRP serum levels (mg/dl)	1.0 ± 1.2
Concurrent medications	
Beta-blocker	65 (100%)
Aspirin	65 (100%)
Nitrates	2 (3%)
ACE inhibitors	37 (57%)
Statins	53 (82%)
Calcium channel blocker	2 (3%)

Data are presented as the mean value ± SD or number (%) of patients.
ACE = angiotensin-converting enzyme; BP = blood pressure; CRP = C-reactive protein; HDL = high-density lipoprotein; IL = interleukin; LDL = low-density lipoprotein.

(<1.5 ml of 2% mepivacaine; Astra Pharmaceuticals) and sterile conditions, a 22-gauge catheter (Braun-Melsungen, Germany) was inserted into the brachial artery of the nondominant arm (left in most cases) for the infusion of drugs or saline. This arm was elevated above the level of the right atrium. All patients were allowed to rest for 20 min after catheter placement to achieve stable baseline measurements before data collection. Forearm blood flow (ml/min per 100 ml forearm tissue) was measured by venous occlusion plethysmography (model EC-4; D. E. Hokanson, Bellevue, Washington) with calibrated mercury-in-Silastic strain gauges applied to the widest part of the forearm. Upper arm cuffs were intermittently inflated to 40 mm Hg for 10 s every 15 s to temporarily prevent venous outflow (Rapid cuff inflator E-10; D. E. Hokanson). To exclude hand circulation from the blood flow, a wrist cuff was inflated to suprasystolic pressure. Drug infusions were administered with a constant-rate infusion pump (Braun-Melsungen). Basal measurements were obtained after intra-arterial sodium chloride (0.9%) infusion (rate of 1 ml/min). For the assessment of endothelium-dependent vasodilation, acetylcholine (Ach) (Ciba Vision GmbH, Aschaffenburg, Germany) was infused intra-arterially in increasing dosages of 10 to 50 μg/min, with infusion rates of 0.8 to 1.2 ml/min. Sodium nitroprusside (SNP) (Schwarz Pharma, Monheim, Germany) was infused for assessment of endothelium-independent vasodilation in increasing dosages of 2 to 8 μg/min, with infusion rates of 0.8 to 1.2 ml/min. Each dose was infused for 5 min, and FBF was measured during the last 2 min of the infusion. Each FBF determination consisted of at least three separate measurements at 15-s intervals. Analysis of the plethysmographic recordings was performed by a technician (Margret Müller-Ardogan), who was unaware of the patients' IL-10 and CRP levels, which were analyzed from blood drawings taken immediately before the FBF measurements.

Laboratory analysis. Serum IL-10 concentrations were measured using a high-sensitivity, quantitative sandwich enzyme immunoassay (Quantikine HS, R&D Systems Europe Ltd., Abingdon, United Kingdom). The lower limit of detection was 0.7 pg/ml, and intra-assay variation among triplicates was 7.6%. High-sensitivity CRP serum levels were measured with an ultrasensitive CRP test (N Latex CRP mono; Behring, Marburg, Germany). The measurement range is 0.02 to 1.1 mg/dl (for 1:20 dilution; higher concentrations were determined after appropriate dilution) with an intra-assay coefficient of variation of 1.7% to 2.5% and an interassay coefficient of variation of 1.7% to 3.6%.

Statistical analysis. Data are expressed as the mean value ± SD. Continuous variables were tested for normal distribution with the Kolmogorov-Smirnov test and compared by one-way analysis of variance (ANOVA). Categorical variables were compared by the chi-square test. In the case of non-normal distribution, the Mann-Whitney *U* test was used. Differences between the group FBF measurements are presented as the mean value ± SEM. Differences in forearm vascular reactivity were examined by repeated measures ANOVA. The FBF responses to ACh and SNP were calculated as the area under the curve and expressed in arbitrary units. Linear regression analysis and nonparametric bivariate correlation (Spearman rank correlation coefficient) were used to compare FBF responses with CRP or IL-10 serum levels. Multivariate analysis was performed with the linear regression model. Serum levels of IL-10 and CRP, as well as low-density lipoprotein (LDL) and high-density lipoprotein (HDL) cholesterol, were included as continuous variables; the clinical characteristics and medication were included as categorical variables. Statistical significance was assumed, if a null hypothesis could be rejected at $p < 0.05$. All statistical analyses were performed with SPSS for Windows 11.0 (SPSS Inc.).

RESULTS

The clinical characteristics of the patient population are summarized in Table 1. Interleukin-10 serum levels ranged from 0.17 to 9.63 pg/ml (median 1.64). There was no correlation between IL-10 and CRP serum levels ($r = 0.08$, $p = 0.5$). As illustrated in Figure 1, ACh-stimulated FBF responses were significantly correlated with IL-10 serum levels ($r = 0.31$, $p < 0.02$). However, despite ACh-stimulated FBF responses being significantly correlated with serum levels of IL-10, FBF responses varied considerably within the lower range of IL-10 serum levels. Because a potential protective function of anti-inflammatory cytokines on endothelial activation may be most prominent in patients with inflammatory activation, we dichotomized the patient population into two groups using the median CRP (median 0.61 mg/dl) serum level as a cut-off value. As

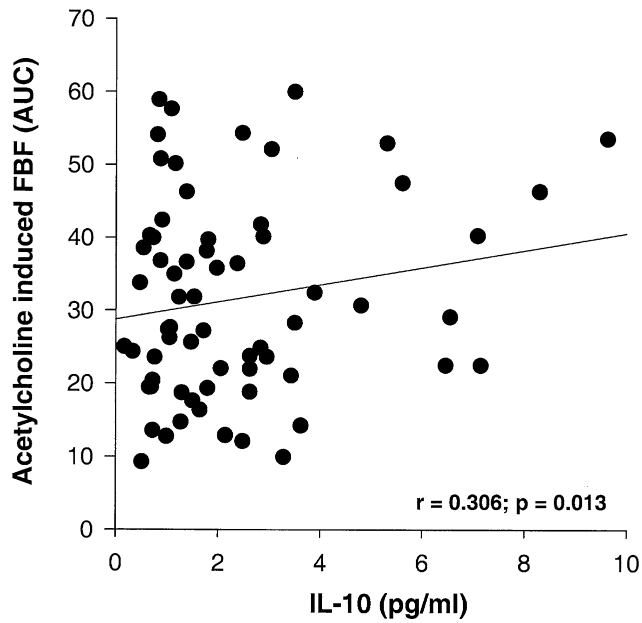

Figure 1. Correlation between and acetylcholine-induced forearm blood flow (FBF) and interleukin (IL)-10 serum levels responses (calculated as the area under the curve [AUC]).

illustrated in Table 2, patients with elevated CRP levels more frequently presented with unstable angina, had higher levels of LDL serum cholesterol, and were less likely to receive treatment with ACE inhibitors. In patients with low CRP serum levels, IL-10 serum levels did not correlate with the ACh-stimulated FBF responses ($r = 0.03$, p = NS). In contrast, there was a significant positive association between IL-10 serum levels and ACh-stimulated FBF responses in patients with elevated serum levels of IL-10 ($r = 0.46$, $p < 0.01$). Thus, increased serum levels of the anti-inflammatory cytokine IL-10 appear to specifically contribute to improved endothelial vasodilator function in patients with enhanced inflammatory activation, as evidenced by augmented CRP serum levels. The FBF responses to the endothelium-independent dilator SNP did not correlate with IL-10 serum levels in either group ($r = 0.12$, $p = 0.33$). In order to further corroborate these findings, we stratified our

Table 2. Patient Characteristics According to Their CRP Serum Levels

	CRP < Median (n = 33)	CRP ≥ Median (n = 32)	p Value
Age (yrs)	52.7 ± 5.6	55.1 ± 2.6	NS
Hypertension	14	15	NS
Diabetes	4	4	NS
Active smoker	8	9	NS
Unstable angina	5	11	<0.05
ACE inhibitors	23	14	<0.05
Statin therapy	29	24	NS
LDL cholesterol (mg/dl)	111.4 ± 31.1	131.3 ± 39.2	<0.05
HDL cholesterol (mg/dl)	45.1 ± 14.3	49.4 ± 13.8	NS

Data are presented as the mean value ± SD or number of patients.
Abbreviations as in Table 1.

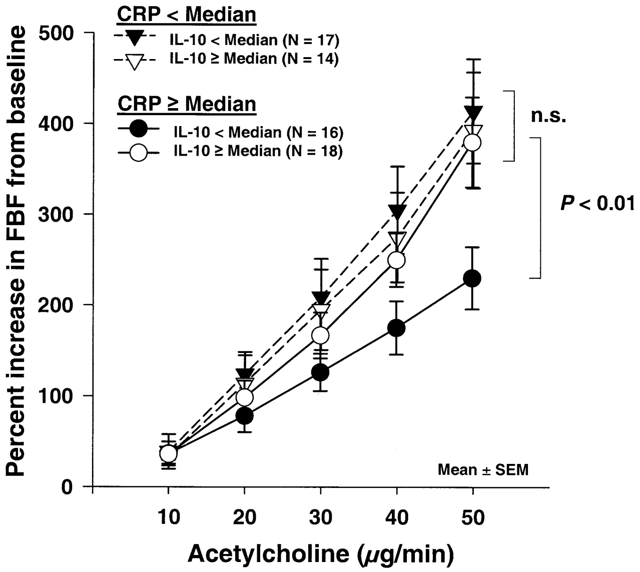

Figure 2. Acetylcholine-induced dose-response curves for forearm blood flow (FBF) calculated as the percent increase in FBF from baseline in patients with C-reactive protein (CRP) less than the median value **(triangles)** and patients with CRP greater than or equal to the median value **(circles)**. **Solid symbols** represent patients with IL-10 serum levels less than the median, and **open symbols** represent patients with IL-10 serum levels greater than or equal to the median. Data are expressed as the mean value ± SEM.

patient cohort into four groups according to their median serum levels of IL-10 and CRP. Patients with low CRP serum levels had similar ACh-stimulated FBF responses, regardless of whether IL-10 serum levels were low or elevated (Fig. 2). In contrast, patients with elevated CRP serum levels exhibited significantly blunted ACh-stimulated FBF responses in the presence of low IL-10 serum levels, whereas patients with elevated IL-10 serum levels showed no significant differences in their FBF, as compared with patients with low CRP serum levels.

Finally, to investigate whether serum levels of IL-10 and high-sensitivity CRP are independent predictors of ACh-stimulated FBF responses, we performed a multivariate analysis including the classic risk factors for CAD, LDL and HDL serum cholesterol levels, clinical symptomatology (stable vs. unstable CAD), ACE inhibitor and statin treatment, as well as CRP and IL-10 serum levels. As illustrated in Table 3, CRP and IL-10 serum levels remained the only statistically significant independent predictors of ACh-stimulated FBF responses in patients with documented CAD. When restricting the multivariate analysis only to the variables that were significant in the univariate model, both serum markers remained independent predictors of ACh-induced FBF. Furthermore, as atherosclerotic disease activity (stable vs. unstable angina) might interfere with serum levels of IL-10 and CRP, we performed an additional multivariate analysis restricted to patients with stable CAD ($n = 49$). This analysis revealed identical results, with IL-10 ($p = 0.016$) and CRP serum levels ($p = 0.030$) remaining

Table 3. Multivariate Analysis, Including Risk Factors for Coronary Artery Disease and Clinical Presentation for Acetylcholine-Induced Forearm Blood Flow (Dependent Variable)

		Multivariate Model		
		Nonstandardized Coefficients		
	Univariate p Value	B	Standard Error	p Value
IL-10 (mg/dl)	0.013	0.99	0.33	0.010
CRP (mg/dl)	0.002	−3.52	−0.37	0.012
LDL cholesterol (mg/dl)	0.49	−0.06	−0.11	0.37
HDL cholesterol (mg/dl)	0.75	0.14	0.08	0.54
Hypertension	0.79	−1.05	−0.05	0.73
Diabetes	0.43	4.32	0.14	0.28
Current smoking	0.82	2.30	0.01	0.92
Unstable angina	0.81	1.90	−0.09	0.53
ACE inhibitors	0.37	0.68	0.01	0.84
Statins	0.91	1.11	0.02	0.58

Adjusted $R^2 = 0.27$. Significance for the model (analysis of variance): $p < 0.05$. Abbreviations as in Table 1.

the only independent predictors of ACh-stimulated FBF responses in patients with stable CAD.

DISCUSSION

The results of the present study demonstrate that serum levels of the anti-inflammatory cytokine IL-10 are independent predictors of the endothelium-mediated vasodilator response of the forearm circulation in patients with CAD. Most importantly, elevated IL-10 serum levels counteract the impairment in systemic endothelial function associated with increased CRP serum levels. These data, for the first time, demonstrate that the balance between pro- and anti-inflammatory cytokines is a major determinant of endothelial function in patients with CAD.

Interleukin-10, which is predominantly secreted by activated monocytes, macrophages, and lymphocytes (16), has multifaceted anti-inflammatory properties, including inhibition of the prototypic inflammatory transcription factor nuclear factor kappa B, leading to suppressed cytokine production (17), inhibition of matrix-degrading metalloproteinases (18), reduction of tissue factor expression (19), inhibition of apoptosis of macrophages and monocytes after infection (20), and promotion of the phenotypic switch of lymphocytes into the Th2 phenotype (21). Most notably with respect to the present study, IL-10 was experimentally shown to protect endothelial function after an acute inflammatory stimulus by limiting local increases in superoxide generation within the vascular wall (15). Likewise, IL-10 was experimentally shown to impede endothelial dysfunction during development of diabetes via reducing the production of superoxide anions (22). Enhanced superoxide anion secretion not only directly scavenges nitric oxide (NO) but also oxidatively modifies cofactors of the nitric oxide synthase (NOS) associated with reduced enzyme activity, resulting in an impaired endothelium dependent vasodilation (23,24). Indeed, in the present study, the protective effects of IL-10 serum levels on endothelial vasodilator function were most prominent in patients with elevated CRP serum levels, which has been shown to directly decrease endothelial NOS expression and increase superoxide anion production in endothelial cells (25–27). Thus, taken together, elevated serum levels of IL-10 might be associated with reduced superoxide anion generation of the activated endothelium in patients with low-grade chronic inflammation of the vascular wall, resulting in improved NO bioavailability and, consequently, increased vasodilator responses to the endothelium-dependent mediator ACh.

Given that endothelial vasodilator function represents a functional read-out of a variety of noxae, as well as protective mediators acting on the vascular wall, the extensive medical treatment of our patient population might have an impact on the results of the present study by unmasking the effects of IL-10 and CRP. Because the cross-sectional design of our study included patients with long-term medical therapy known to modulate endothelial function in the presence of CAD (e.g., statins and ACE inhibitors), we cannot address the question of whether statin or ACE inhibitor treatment alters the association between endothelial vasodilator function and inflammatory markers. Likewise, because women were excluded, we cannot comment on a potential interference with sex hormones. Finally, although our multivariate analysis included all previously established major determinants of endothelial function in patients with CAD, we cannot exclude that subtle differences in risk factor profile or effective treatment might have gone unnoticed. However, regardless of these potential limitations, the results of the present study, obtained in a real-world scenario of patients extensively treated with aspirin, beta-blockers, statins, and ACE inhibitors, support the concept of further enhancement of an anti-inflammatory mechanism in order to improve endothelial vasodilator function, especially in patients with elevated CRP serum levels.

Heitzer et al. (11) have previously shown that the improvement in systemic endothelial vasodilator function by acutely scavenging reactive oxygen species, using intra-arterial infusion of vitamin C, is a major determinant of atherosclerotic disease progression in patients at risk. The data of the present study now further support the concept that the balance between pro- and anti-inflammatory cytokines is a major determinant of endothelial vasodilator function in patients with CAD. Moreover, our previous studies have demonstrated that elevated IL-10 serum levels abrogated the increased risk of death and recurrence of myocardial infarction associated with elevated CRP levels and independently predicted the clinical outcome in patients with ACS (5), thereby extending a previous report suggesting an association between elevated IL-10 serum levels and decreased risk of coronary events in patients with unstable angina (28). Most importantly, recent experimental studies could demonstrate that systemic or local IL-10 gene transfer not only attenuates atherogenesis (16,29,30) but also modulates processes associated with lesion progression (21). Because endothelial vasodilator function integrates the risk factor load on the vascular wall (8) and represents an established surrogate marker for atherosclerotic disease progression in patients with CAD (10) or at risk of developing atherosclerosis (9,11,31), it will be important to disclose whether therapeutic interventions that increase circulating IL-10 levels may improve endothelial vasodilator function in patients with CAD and ongoing elevated inflammatory activity.

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Reprint requests and correspondence: Dr. Stephan Fichtlscherer, Department of Internal Medicine IV, Division of Cardiology, J. W. Goethe University, Theodor-Stern-Kai 7, D-60590 Frankfurt, Germany. E-mail: fichtlscherer@em.uni-frankfurt.de.

REFERENCES

1. Ross R. Atherosclerosis—an inflammatory disease. N Engl J Med 1999;340:115–26.
2. Libby P, Ridker PM. Novel inflammatory markers of coronary risk: theory versus practice. Circulation 1999;100:1148–50.
3. Ridker PM, Cushman M, Stampfer MJ, Tracy RP, Hennekens CH. Inflammation, aspirin, and the risk of cardiovascular disease in apparently healthy men. N Engl J Med 1997;336:973–9.
4. Ridker PM, Rifai N, Pfeffer MA, et al. Inflammation, pravastatin, and the risk of coronary events after myocardial infarction in patients with average cholesterol levels. Circulation 1998;98:839–44.
5. Heeschen C, Dimmeler S, Hamm CW, et al. Serum level of the antiinflammatory cytokine interleukin-10 is an important prognostic determinant in patients with acute coronary syndromes. Circulation 2003;107:2109–14.
6. Tomai F, Crea F, Gaspardone A, et al. Unstable angina and elevated C-reactive protein levels predict enhanced vasoreactivity of the culprit lesion. Circulation 2001;104:1471–6.
7. Fichtlscherer S, Rosenberger G, Walter DH, Breuer S, Dimmeler S, Zeiher AM. Elevated C-reactive protein levels and impaired endothelial vasoreactivity in patients with coronary artery disease. Circulation 2000;102:1000–6.
8. Bonetti PO, Lerman LO, Lerman A. Endothelial dysfunction: a marker of atherosclerotic risk. Arterioscler Thromb Vasc Biol 2003; 23:168–75.
9. Suwaidi JA, Hamasaki S, Higano ST, Nishimura RA, Holmes DR, Lerman A. Long-term follow-up of patients with mild coronary artery disease and endothelial dysfunction. Circulation 2000;101:948–54.
10. Schächinger V, Britten MB, Zeiher AM. Prognostic impact of coronary vasodilator dysfunction on adverse long-term outcome of coronary heart disease. Circulation 2000;101:1899–906.
11. Heitzer T, Schlinzig T, Krohn K, Meinertz T, Münzel T. Endothelial dysfunction, oxidative stress, and risk of cardiovascular events in patients with coronary artery disease. Circulation 2001;104:2673–8.
12. Perticone F, Ceravolo R, Pujia A, et al. Prognostic significance of endothelial dysfunction in hypertensive patients. Circulation 2001; 104:191–6.
13. Halcox JPJ, Schenke WH, Zalos G, et al. Prognostic value of coronary vascular endothelial dysfunction. Circulation 2002;106:653–8.

14. Gokce N, Keaney JF Jr., Hunter LM, Watkins MT, Menzoian JO, Vita JA. Risk stratification for postoperative cardiovascular events via noninvasive assessment of endothelial function: a prospective study. Circulation 2002;105:1567–72.
15. Gunnett CA, Heistad DD, Berg DJ, Faraci FM. IL-10 deficiency increases superoxide and endothelial dysfunction during inflammation. Am J Physiol (Heart Circ Physiol) 2000;279:H1555–62.
16. Mallat Z, Besnard S, Duriez M, et al. Protective role of interleukin-10 in atherosclerosis. Circ Res 1999;85:E17–24.
17. Wang P, Wu P, Siegel MI, Egan RW, Billah MM. Interleukin (IL)-10 inhibits nuclear factor kappa B (NF kappa B) activation in human monocytes. IL-10 and IL-4 suppress cytokine synthesis by different mechanisms. J Biol Chem 1995;270:9558–63.
18. Lacraz S, Nicod LP, Chicheportiche R, Welgus HG, Dayer JM. IL-10 inhibits metalloproteinase and stimulates TIMP-1 production in human mononuclear phagocytes. J Clin Invest 1995;96:2304–10.
19. Lindmark E, Tenno T, Chen J, Siegbahn A. IL-10 inhibits LPS-induced human monocyte tissue factor expression in whole blood. Br J Haematol 1998;102:597–604.
20. Arai T, Hiromatsu K, Nishimura H, et al. Endogenous interleukin 10 prevents apoptosis in macrophages during *Salmonella* infection. Biochem Biophys Res Commun 1995;213:600–7.
21. Pinderski LJ, Fischbein MP, Subbanagounder G, et al. Overexpression of interleukin-10 by activated T lymphocytes inhibits atherosclerosis in LDL receptor-deficient mice by altering lymphocyte and macrophage phenotypes. Circ Res 2002;90:1064–71.
22. Gunnett CA, Heistad DD, Faraci FM. Interleukin-10 protects nitric oxide-dependent relaxation during diabetes: role of superoxide. Diabetes 2002;51:1931–7.
23. Heitzer T, Brockhoff C, Mayer B, et al. Tetrahydrobiopterin improves endothelium-dependent vasodilation in chronic smokers: evidence for a dysfunctional nitric oxide synthase. Circ Res 2000;86:E36–41.
24. Landmesser U, Dikalov S, Price SR, et al. Oxidation of tetrahydrobiopterin leads to uncoupling of endothelial cell nitric oxide synthase in hypertension. J Clin Invest 2003;111:1201–9.
25. Verma S, Wang CH, Li SH, et al. A self-fulfilling prophecy: C-reactive protein attenuates nitric oxide production and inhibits angiogenesis. Circulation 2002;106:913–9.
26. Venugopal SK, Devaraj S, Yuhanna I, Shaul P, Jialal I. Demonstration that C-reactive protein decreases eNOS expression and bioactivity in human aortic endothelial cells. Circulation 2002;106:1439–41.
27. Wang C-H, Li S-H, Weisel RD, et al. C-reactive protein upregulates angiotensin type 1 receptors in vascular smooth muscle. Circulation 2003;107:1783–90.
28. Anguera I, Miranda-Guardiola F, Bosch X, et al. Elevation of serum levels of the anti-inflammatory cytokine interleukin-10 and decreased risk of coronary events in patients with unstable angina. Am Heart J 2002;144:811–7.
29. Pinderski Oslund LJ, Hedrick CC, Olvera T, et al. Interleukin-10 blocks atherosclerotic events in vitro and in vivo. Arterioscler Thromb Vasc Biol 1999;19:2847–53.
30. von der Thusen JH, Kuiper J, Fekkes ML, de Vos P, van Berkel TJC, Biessen EAL. Attenuation of atherogenesis by systemic and local adenovirus-mediated gene transfer of interleukin-10 in LDLr −/− mice. FASEB J 2001;15:2730–2.
31. Targonski PV, Bonetti PO, Pumper GM, Higano ST, Holmes DR Jr., Lerman A. Coronary endothelial dysfunction is associated with an increased risk of cerebrovascular events. Circulation 2003;107:2805–9.